



University of Groningen

## SecYEG proteoliposomes catalyze the Delta psi-dependent membrane insertion of FtsQ

van der Laan, M.; Nouwen, N.; Driessen, A.J.M.

*Published in:*  
The Journal of Biological Chemistry

*DOI:*  
[10.1074/jbc.m306527200](https://doi.org/10.1074/jbc.m306527200)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2004

[Link to publication in University of Groningen/UMCG research database](#)

### *Citation for published version (APA):*

van der Laan, M., Nouwen, N., & Driessen, A. J. M. (2004). SecYEG proteoliposomes catalyze the Delta psi-dependent membrane insertion of FtsQ. *The Journal of Biological Chemistry*, 279(3), 1659 - 1664.  
<https://doi.org/10.1074/jbc.m306527200>

### **Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

### **Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

## SecYEG Proteoliposomes Catalyze the $\Delta\psi$ -Dependent Membrane Insertion of FtsQ\*

Received for publication, June 19, 2003, and in revised form, October 24, 2003  
Published, JBC Papers in Press, October 25, 2003, DOI 10.1074/jbc.M306527200

Martin van der Laan, Nico Nouwen, and Arnold J. M. Driessen‡

From the Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute,  
University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

**In *Escherichia coli*, the insertion of most inner membrane proteins is mediated by the Sec translocase. Ribosome-bound nascent chains of Sec-dependent inner membrane proteins are targeted to the SecYEG complex via the signal recognition particle pathway. We now demonstrate that the signal recognition particle-dependent co-translational membrane targeting and membrane insertion of FtsQ can be reconstituted with proteoliposomes containing purified SecYEG. SecA and a transmembrane electrical potential are essential for the translocation of the large periplasmic domain of FtsQ, whereas co-reconstituted YidC has an inhibitory effect. These data demonstrate that membrane protein insertion can be reconstituted with a minimal set of purified Sec components.**

In *Escherichia coli*, translocase mediates the transport of secretory proteins across as well as the insertion of most membrane proteins into the inner membrane (reviewed in Refs. 1 and 2). Although secretory proteins are post-translationally targeted to the translocase, inner membrane proteins (IMPs)<sup>1</sup> generally employ the co-translational SRP pathway (reviewed in Ref. 3). SRP recognizes hydrophobic signal sequences or transmembrane segments (TMS) in nascent IMPs. Upon interaction of SRP with its membrane-bound receptor, FtsY, and subsequent GTP hydrolysis, nascent IMPs are transferred to the protein-conducting SecYEG complex (4, 5). Insertion of TMS can occur in the absence of SecA (5, 6), whereas translocation of large periplasmic domains seems to require SecA (7–10). Recently, an integral membrane protein, YidC, has been identified that can be cross-linked to SecYEG-dependent IMPs during their insertion (11–13). Depletion of YidC in *E. coli* moderately affects the insertion of Sec-dependent IMPs but strongly inhibits Sec-independent membrane protein insertion *in vivo* (14).

SecYEG-mediated translocation of preproteins has been reconstituted from purified components (15). Recently, we have reconstituted the initial steps of IMP insertion with SecYEG/YidC proteoliposomes (16). Membrane-inserted nascent chains of the monotopic membrane protein FtsQ cross-linked to SecY and YidC, but the reconstituted system did not support mem-

brane insertion of full-length FtsQ, which includes translocation of the C-terminal periplasmic domain. We now report on the reconstitution of the complete membrane insertion of FtsQ using SecYEG proteoliposomes. Membrane insertion requires the SRP targeting pathway, a transmembrane electrical potential ( $\Delta\psi$ ), and SecA but not YidC.

### EXPERIMENTAL PROCEDURES

**Strains and Plasmids**—Wild-type inverted inner membrane vesicles (IMVs) were prepared from *E. coli* SF100 (17), which was also used for overexpression of SecYEG (18) and YidC (16). SecYE was overexpressed in strain *E. coli* NN104, a  $\Delta secG$  derivative of SF100. Strain NN100 (19) was used to obtain IMVs lacking  $F_1F_0$  ATPase. YidC depletion strain JS7131 (14) was grown for 3.5 h in the presence of either 0.1% glucose or arabinose for the preparation of YidC-depleted and control IMVs, respectively. Strain MC4100 was used to obtain S135 lysate. Plasmid pBSKftsQ was constructed by ligating the BamHI/HindIII fragment of pNB1 (21) into BamHI/HindIII-cut pC4meth101FtsQ (22).

**In Vitro Transcription, Translation, and Insertion Reaction**—The RiboMax *in vitro* transcription kit (Promega) with plasmid pBSKftsQ as template was used for the synthesis of *ftsQ* mRNA. *In vitro* translation reactions were carried out for 30 min at 37 °C in the presence of the indicated amounts of IMVs or proteoliposomes as described by de Vrije *et al.* (23). Reactions were started by the addition of lysate. To generate a  $\Delta\psi$ , the  $K^+$  concentration in the reaction mixture was adjusted to 100 mM, and valinomycin was added at 0.5  $\mu$ M. After 30 min at 37 °C, a small sample of the reaction mixture was removed as synthesis control, and the remainder was treated with 0.4 mg/ml proteinase K for 30 min on ice in the presence or absence of 1% Triton X-100 as indicated. Samples were trichloroacetic acid-precipitated and analyzed by 12% SDS-PAGE and phosphorimaging and quantified using the LumiAnalyst software from Roche Applied Science. For carbonate extraction, reactions were split in half, and liposomes or SecYEG proteoliposomes were isolated from the mixture by centrifugation. Pellets were resuspended in 50 mM HEPES-KOH, pH 8.0, 0.2 mg/ml proteinase K or in 0.2 M  $Na_2CO_3$ , pH 11.2.

**Other Methods**—SecA (24), FtsY (25), SecYE(G) (18), PrlA4 (18, 20), and YidC (16) were purified as described. Reconstitutions were carried out as described (16). For  $\Delta\psi$  generation, the reconstitution buffer contained 100 mM  $Na_2SO_4$  or 100 mM KCl. Where indicated, SecA or FtsY was removed from the lysate by immunodepletion (26), and depletion was verified by immunoblotting. Proton motive force (PMF) measurements in IMVs were performed using the fluorescent dye 9-amino-6-chloro-2-methoxyacridin (19).

### RESULTS

**Co-translational Insertion of FtsQ into Wild-type IMVs**—To study the insertion of full-length FtsQ into IMVs, a co-translational targeting/insertion assay was developed in which the translocation of the periplasmic domain of FtsQ is monitored by protease protection. If FtsQ is inserted in the correct orientation, the periplasmic C-terminal domain is located in the lumen of the IMVs and therefore is not accessible to externally added proteases. The short cytoplasmic N-terminal tail of FtsQ that remains on the outside of the IMVs will be degraded (Fig. 1A). The resultant truncation of about 24 amino acids of FtsQ can be detected on SDS-PAGE. IMPs have been shown to be

\* This work was supported by the Earth and Life Sciences Foundation, which is subsidized by the Netherlands Organization for Scientific Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 31-50-3632164; Fax: 31-50-3632154; E-mail: a.j.m.driessen@biol.rug.nl.

<sup>1</sup> The abbreviations used are: IMP, inner membrane protein; IMV, inner membrane vesicle; SRP, signal recognition particle; TMS, transmembrane segment; PMF, proton motive force.

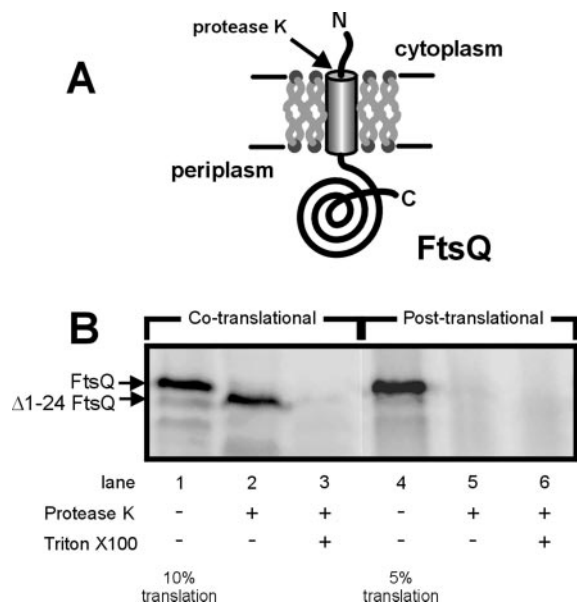


FIG. 1. Schematic representation of the model protein FtsQ (A) and the co- and post-translational insertion of FtsQ into IMVs (B). In the co-translational reaction, *ftsQ* mRNA was translated in the presence of 25  $\mu$ g of SF100 IMVs (lane 1, 10% of the total translation). Samples were subsequently treated with proteinase K in the absence (lanes 2 and 5) or presence of 1% Triton X-100 (lanes 3 and 6). In the post-translational reaction, synthesis was performed in the absence of IMVs (lane 4, 5% of the total translation), translation was stopped by the addition of 25  $\mu$ g/ml chloramphenicol. After 5 min, IMVs were added, and samples were incubated for 20 min at 37 °C.

targeted to the translocase and membrane-inserted in a co-translational manner (2). Indeed, when FtsQ was synthesized in the presence of IMVs, the protein was properly membrane-inserted (Fig. 1B, lanes 1–3). For post-translational translocation, FtsQ was synthesized in the absence of membranes, whereupon further translation was blocked with chloramphenicol before IMVs were added (Fig. 1B, lanes 4–6). Under these conditions, no insertion of FtsQ was observed indicating a strictly co-translational process. In contrast, the secretory protein proOmpA was translocated efficiently under these conditions (data not shown). The co-translational assay was used to investigate the minimal requirements for the membrane insertion of FtsQ.

**The Proton Motive Force Is Required for Membrane Insertion of FtsQ**—The PMF has been shown to play an important role in the insertion of some IMPs (27) and membrane topology control (28, 29). The ATP needed for the translation reaction is also used by the  $F_1F_0$  ATPase to generate a PMF. When PMF generation was inhibited by the addition of the ionophores valinomycin and nigericin, membrane insertion of full-length FtsQ was almost completely blocked (Fig. 2A). A similar result was obtained with IMVs prepared from *E. coli* strain NN100, in which the *atp* operon is deleted. These IMVs do not generate a PMF with ATP (Ref. 19; data not shown). Insertion of FtsQ into NN100 IMVs was strongly reduced compared with wild-type IMVs (Fig. 2). However, when sodium succinate was added to allow PMF generation via the respiratory chain, insertion activity was restored (Fig. 2B). These data demonstrate that the PMF is required for membrane insertion of full-length FtsQ.

**Depletion of YidC Has No Effect on Membrane Insertion of FtsQ**—It has been suggested that YidC facilitates the release of TMS from the translocase into the lipid bilayer (2). Membrane-inserted nascent FtsQ can be cross-linked to reconstituted YidC in a SecYEG-dependent manner (16), but *in vivo* YidC depletion experiments indicate that YidC is not essential for the membrane insertion of FtsQ (9). YidC-depleted IMVs, pre-

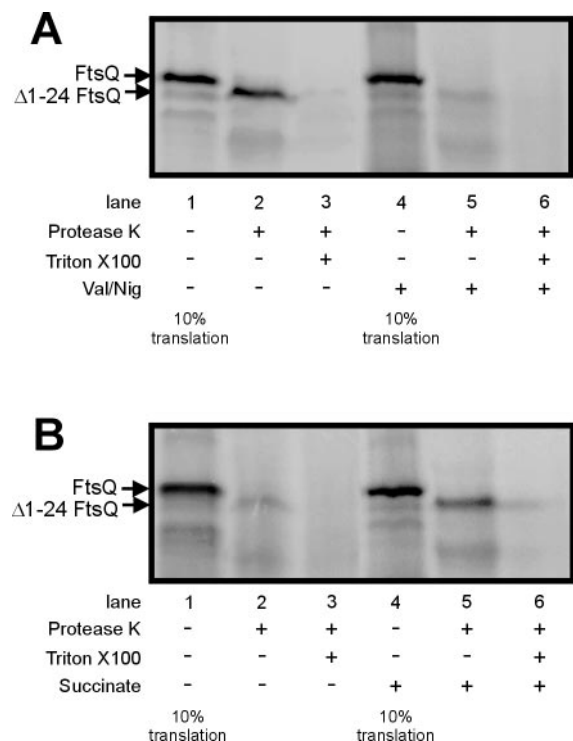


FIG. 2. The PMF is required for membrane insertion of FtsQ. A, insertion into wild-type IMVs was carried out as described in the legend to Fig. 1 in the absence (lanes 1–3) or presence (lanes 4–6) of 1  $\mu$ M valinomycin (Val) and 1  $\mu$ M nigericin (Nig). B, insertion into IMVs lacking  $F_1F_0$  ATPase was performed in the absence (lanes 1–3) or presence (lanes 4–6) of 5 mM sodium succinate.

pared as described previously (30), are devoid of immunodelectable YidC. Consistent with the *in vivo* studies, indeed, membrane insertion of FtsQ into wild-type and YidC-depleted IMVs occurred with comparable efficiency (Fig. 3A). Interestingly, overexpression of YidC in strain SF100 inhibited membrane insertion of FtsQ (Fig. 3B), whereas it had little effect on the post-translational translocation of proOmpA (data not shown). Because PMF generation with ATP *in vitro* was not affected by overexpression of YidC (data not shown), it appears that YidC is directly responsible for the observed inhibition.

**Reconstitution of SecA- and PMF-dependent FtsQ Insertion Using SecYEG Proteoliposomes**—Overproduction of SecYEG was found to be sufficient to increase the efficiency of FtsQ insertion (Fig. 3C), suggesting that SecYEG and the PMF are the minimal requirements for the correct membrane insertion of FtsQ. Ribosome-bound nascent FtsQ inserts into SecYEG proteoliposomes in the absence of a PMF (5, 16), but insertion of full-length FtsQ could not be detected under these conditions. To test the possibility that the PMF was the main limiting factor, insertion assays were conducted with SecYEG proteoliposomes in which a transmembrane electrical potential ( $\Delta\psi$ ) was imposed by means of an inwardly directed valinomycin-mediated  $K^+$  diffusion gradient. This results in the formation of a physiologically oriented  $\Delta\psi$ , *i.e.* inside positive. Indeed, when sodium-loaded SecYEG proteoliposomes were diluted into a potassium-containing buffer in the presence of valinomycin to generate a  $\Delta\psi$ , inside positive, efficient membrane insertion of FtsQ was observed (Fig. 4B). When potassium-loaded SecYEG proteoliposomes were used instead, no membrane insertion of FtsQ was observed, which demonstrates a strict requirement for a  $\Delta\psi$ . The membrane insertion activity strictly depends on the presence of SecYEG, as protein-free liposomes did not support any significant protease protection either in the absence or presence of a  $\Delta\psi$  (Fig. 4A).

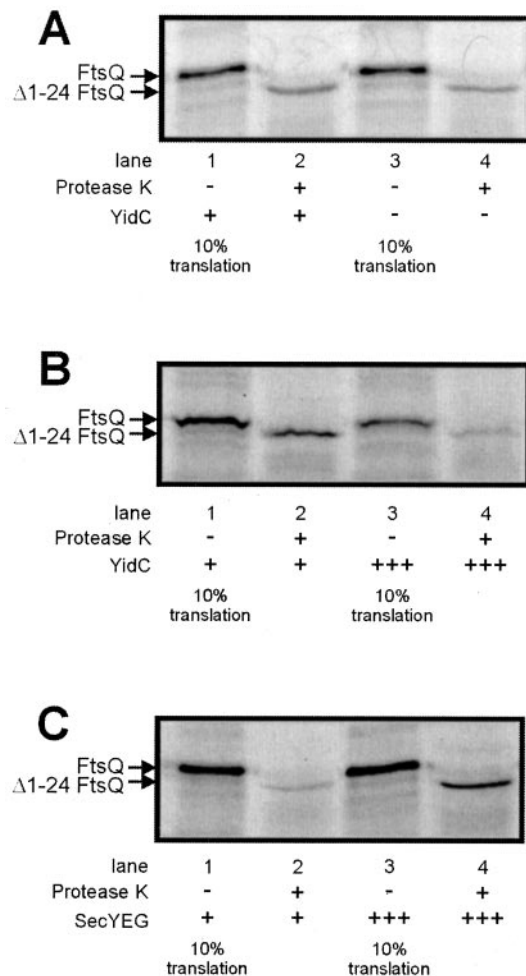


FIG. 3. Depletion of YidC has hardly any effect on the membrane insertion of FtsQ. **A**, reactions were carried out in the presence of wild-type *E. coli* JS7131 (lanes 1 and 2) or YidC-depleted JS7131 (lanes 3 and 4) IMVs. **B**, overexpression of YidC inhibits membrane insertion of FtsQ. Insertion reactions were performed with 25  $\mu$ g of wild-type SF100 (lanes 1 and 2) or YidC-overproducing SF100 IMVs (lanes 3 and 4). **C**, overexpression of SecYEG strongly stimulates membrane insertion of FtsQ. Insertion reactions contained 2.5  $\mu$ g of wild-type SF100 (lanes 1 and 2) or SecYEG-overproducing SF100 IMVs (lanes 3 and 4).

*In vivo* experiments suggest that the translocation of the periplasmic domain of FtsQ requires SecA (9). On the other hand, initial membrane insertion of nascent FtsQ does not depend on SecA, although these translation-arrested nascent chains can be cross-linked to SecA (4, 11). Because the translation lysate contains wild-type levels of SecA, SecYEG proteoliposomes were tested with a lysate that was immunodepleted from SecA.  $\Delta\psi$ -Dependent membrane insertion of FtsQ with this lysate occurred only upon the addition of purified SecA (Fig. 4, C and D). These data demonstrate that both SecA and a PMF are essential for FtsQ membrane insertion.

It is generally believed that Sec-dependent membrane proteins are targeted to the translocase by the SRP pathway (3). Nascent chains of FtsQ have been shown to cross-link to the SRP protein, Ffh (31). Release of nascent chains from SRP and their insertion into the translocase depends on FtsY (4). When the SRP pathway was disrupted by immunodepletion of FtsY from the lysate, insertion of FtsQ into SecYEG proteoliposomes was strongly inhibited (Fig. 4E, lane 2). Membrane insertion efficiency could be restored by the addition of purified FtsY (Fig. 4E, lane 4). These data show that FtsQ is indeed targeted to the Sec translocase via the SRP pathway.

Finally, proteoliposomes containing only SecYE mediate the translocation of secretory proteins, albeit with reduced efficiency (Refs. 32 and 33; data not shown). SecYE proteoliposomes supported only a very low level of FtsQ insertion (Fig. 5, A and B). These results indicate that SecG is required for efficient membrane insertion of FtsQ.

**PMF Dependence of FtsQ Insertion Is Unchanged in the PrlA4 Mutant**—Nouwen *et al.* (34) have shown that in the *prlA4* mutant of SecY translocation of preproteins is less PMF-dependent. Interestingly, membrane insertion of full-length FtsQ is still strictly  $\Delta\psi$ -dependent in PrlA4 proteoliposomes (Fig. 5, A and B), whereas these proteoliposomes catalyze the efficient  $\Delta\psi$ -independent translocation of proOmpA (20, data not shown). These data suggest that the PMF promotes FtsQ insertion by a mechanism different from that found with pre-protein translocation.

**High Amounts of Co-reconstituted YidC Inhibit SecYEG-mediated Membrane Insertion of FtsQ**—As overexpression of YidC inhibited the translocation of the periplasmic domain of FtsQ in IMVs, we co-reconstituted YidC together with SecYEG. A large excess of YidC compared with SecYEG was incorporated into the proteoliposomes to mimic native membranes in which YidC is clearly more abundant than SecYEG (10). Co-reconstituted YidC significantly inhibited SecYEG-mediated membrane insertion of FtsQ (Fig. 5C) without having an effect on translation efficiency. The extent of inhibition was dependent on the amount of co-reconstituted YidC, whereas YidC only marginally affected the co-translational (data not shown) and post-translational (16) translocation of proOmpA into SecYEG proteoliposomes.

**The Transmembrane Segment of FtsQ Is Stably Integrated into the Lipid Bilayer of SecYEG Proteoliposomes**—To determine whether the TMS of FtsQ had left the translocase and been integrated into the lipid bilayer, stable membrane insertion of the TMS was tested by sodium carbonate extraction. When FtsQ was synthesized in the presence of protein-free liposomes, about 20% of the translation product was found associated with membranes. However, the majority of the membrane-associated FtsQ could be extracted with carbonate (Fig. 6A). The small amount of material in the carbonate pellets probably reflects aggregated protein. When  $\Delta\psi$ -generating SecYEG proteoliposomes were used under conditions that allow the efficient translocation of the periplasmic domain of FtsQ, an increase in the amount of membrane-associated FtsQ was observed (Fig. 6B). Moreover, the majority of the membrane-associated FtsQ was not extractable with carbonate, suggesting that the TMS had been stably integrated into the lipid bilayer. In addition, the amount of carbonate-resistant FtsQ in SecYEG proteoliposomes correlated very well with the amount of protease-protected FtsQ, suggesting that the protein is exclusively inserted in the correct topology.

## DISCUSSION

In this paper, we report on the functional reconstitution of SecYEG-mediated membrane protein insertion using FtsQ as a model protein. FtsQ is a monotopic membrane protein with an N-terminal transmembrane domain and a large C-terminal periplasmic domain. Previous studies utilized IMVs as target membranes (12, 35). However, the reconstitution of such a complex process is an important step toward a detailed understanding of the minimal requirements of membrane protein insertion. The reconstituted system now precisely defines these requirements without the risk of the pleiotropic effects often associated with *in vivo* depletion or inhibition experiments. We show that the  $\Delta\psi$  and SecA are essential for the SecYEG-dependent membrane insertion of FtsQ. The mechanistic role of the  $\Delta\psi$  remains to be elucidated. However, a striking observa-



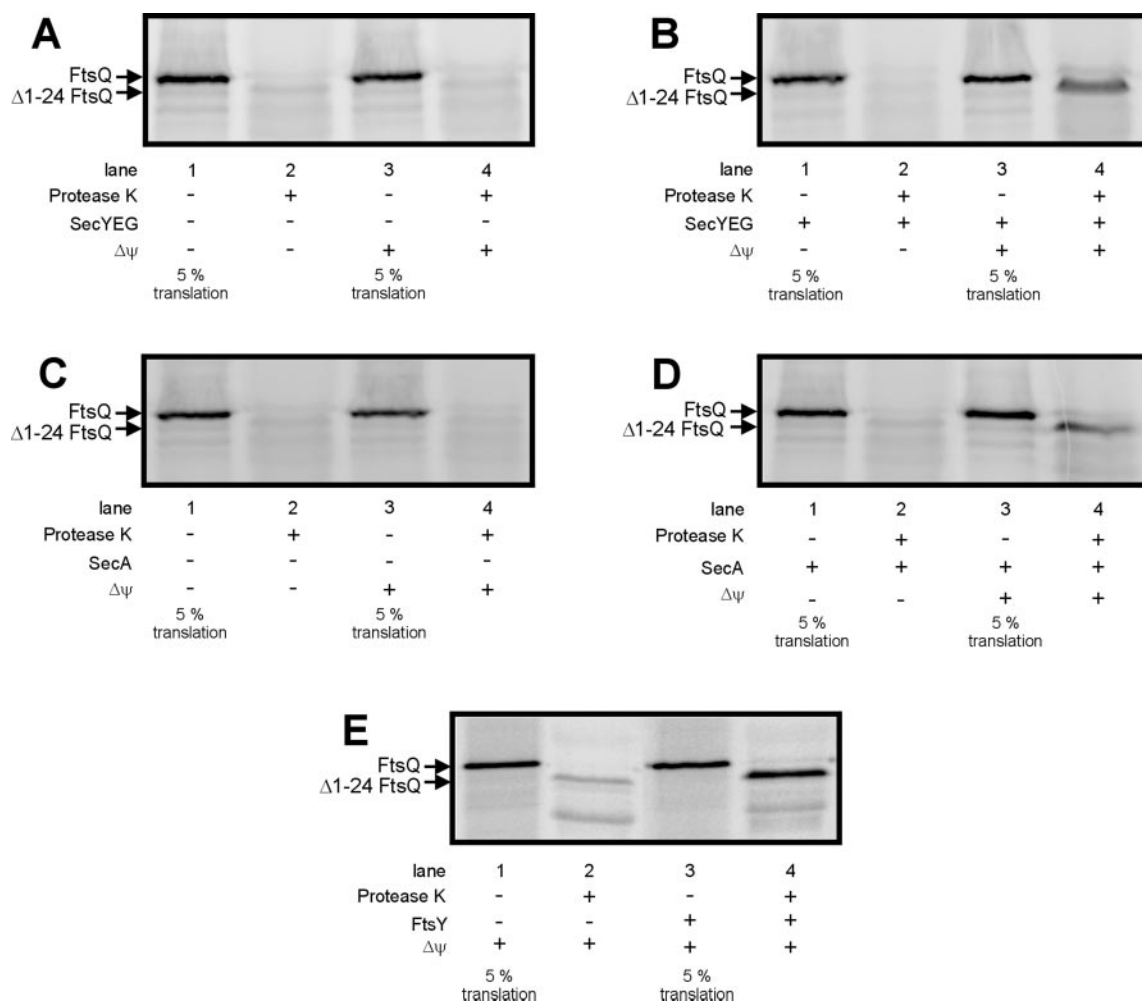


FIG. 4. Insertion of FtsQ into proteoliposomes is dependent on the presence of SecYEG (A and B), SecA (C and D), and FtsY (E) and strictly requires a  $\Delta\psi$  (A–D). A and B, liposomes were prepared from *E. coli* phospholipids and reconstituted with 20  $\mu$ g of purified SecYEG where indicated.  $\text{Na}^+$ -loaded (+ $\Delta\psi$ ) or  $\text{K}^+$ -loaded (– $\Delta\psi$ ) (proteo-)liposomes were diluted into a  $\text{K}^+$ -buffer in the presence of 0.5  $\mu\text{M}$  valinomycin. C and D, translation reactions with SecA-immunodepleted lysate were carried out in the absence (C) or presence of 0.5  $\mu$ g purified SecA (D). E, assays with SecYEG proteoliposomes and FtsY-immunodepleted lysate under  $\Delta\psi$ -generating conditions were carried out in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of 0.5  $\mu$ g of purified FtsY.

tion is that, unlike preprotein translocation (34), FtsQ membrane insertion remained  $\Delta\psi$ -dependent when the PrLA4 mutant of SecY was used. PrLA4 has been shown to suppress the  $\Delta\psi$  requirement of both the initial insertion of the signal sequence (36) as well as the translocation of mature domains (34) of various preproteins. Translocation of the periplasmic domain of FtsQ most likely occurs by a similar mechanism as the translocation of preproteins; therefore, the periplasmic domain of FtsQ is expected to translocate largely PMF-independently in the PrLA4 mutant. Because this is not the case, we suggest that the strict  $\Delta\psi$  requirement for the co-translocational membrane insertion of FtsQ relates to a step different from translocation of the periplasmic domain. The initial membrane insertion of the TMS of nascent FtsQ as revealed by cross-linking studies occurs independently of the PMF (5, 16), but it is not known whether the inserted transmembrane domain in the absence of a  $\Delta\psi$  is already in its correct orientation. Therefore, future studies should be done to determine whether the  $\Delta\psi$  is needed to acquire a proper topology of this hydrophobic segment in order to position the polar periplasmic FtsQ domain for SecA-dependent translocation. Apparently, this requirement cannot be compensated by the PrLA4 mutant, which signifies an important mechanistic difference between the translocation of secretory proteins with a (non-)cleavable signal sequence and the insertion of monotopic membrane proteins.

We also show that SecG is required for the membrane insertion of FtsQ, as SecYE proteoliposomes do not catalyze this reaction. Koch *et al.* (37) have demonstrated that SecG is dispensable for the insertion of the polytopic IMP MtlA. However, MtlA does not contain any large periplasmic domains, and its insertion also is not dependent on SecA. In contrast, membrane insertion of YidC does require SecG as well as SecA (10, 32). SecG has been suggested to modulate the SecA activity (38, 39), which might explain why the requirement for these two proteins seems to go hand in hand.

The reconstituted assay shows a clear FtsY dependence of 1 Sec-dependent membrane protein insertion. This is consistent with an involvement of the SRP pathway in the targeting of nascent FtsQ to the membrane. It should be stressed that IMVs always contain a large amount of FtsY and FtsY fragments that cannot be removed by extraction with urea or sodium carbonate (Ref. 40; data not shown). The proteoliposomes used in this study are entirely free of FtsY, whereas the lysate acts as a source of soluble FtsY. Because the soluble FtsY purified from the cytosol of an overexpression strain is functional in supporting membrane protein insertion, it appears that the co-translocational targeting of FtsY to the inner membrane, as suggested by Herskovits *et al.* (41), to occur *in vivo* is not essential for the FtsY function.

Our results raise additional questions about the role of YidC

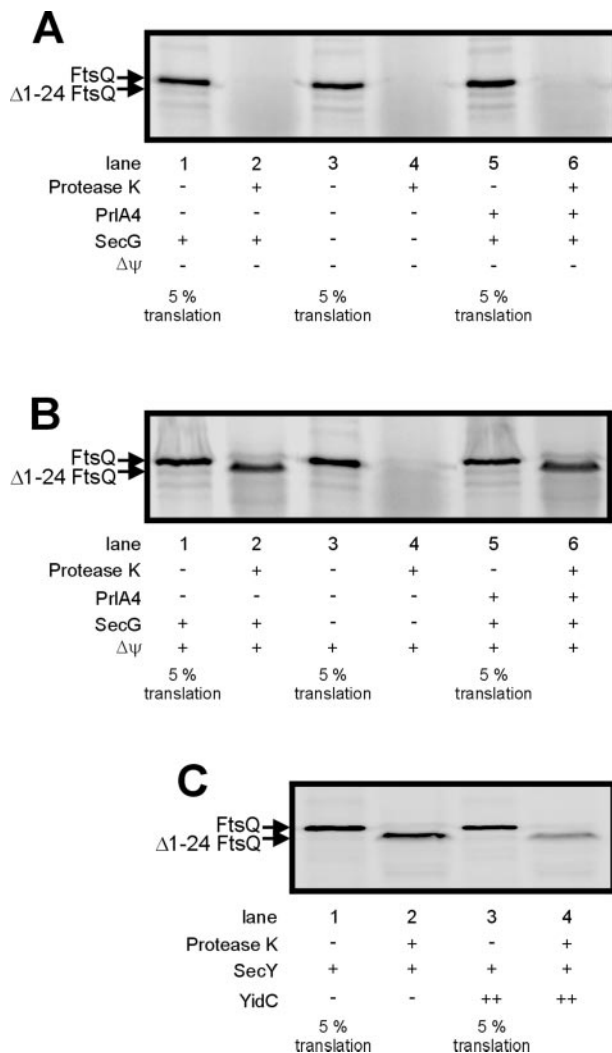


FIG. 5. The PMF dependence of FtsQ translocation is not affected by the PrlA4 mutation in SecY (A and B). Membrane insertion of FtsQ is dependent on SecG (B). Translocation is inhibited by co-reconstituted YidC (C). Proteoliposomes were reconstituted with 20  $\mu$ g of purified SecYE, SecYEG, or PrlA4 complex. Where indicated, 60  $\mu$ g of YidC was co-reconstituted with SecYEG.

in Sec-dependent membrane protein insertion. On the basis of cross-linking studies, it has been suggested that YidC interacts with nascent TMS, whereas a YidC defect *in vivo* seems to cause a pleiotropic effect on protein translocation, probably because of jamming of the translocase (14, 42, 43). It has been postulated that YidC catalyzes the lateral transfer of TMS from the translocase into the lipid bilayer. However, we show that the TMS of FtsQ leaves the translocase and becomes stably integrated into the membrane also in the absence of YidC as judged by the carbonate resistance of the inserted FtsQ. Taken together, for FtsQ membrane insertion, the role of YidC may be restricted to a kinetic effect only. A more general role of YidC as a chaperone in the membrane interacting with hydrophobic TMS (44) would imply that the binding of YidC to nascent proteins may arrest the translocation process transiently. Consequently, elevated levels of YidC would then inhibit translocation as observed in this study. Depletion of YidC, however, did not stimulate FtsQ insertion; however, this experiment is complicated by the fact that membranes derived from YidC-depleted cells are impaired in their capacity to generate a PMF (30). We therefore propose a model in which YidC generally samples the TMS of inserting nascent membrane proteins at the lateral opening of the SecYEG pore. Various membrane

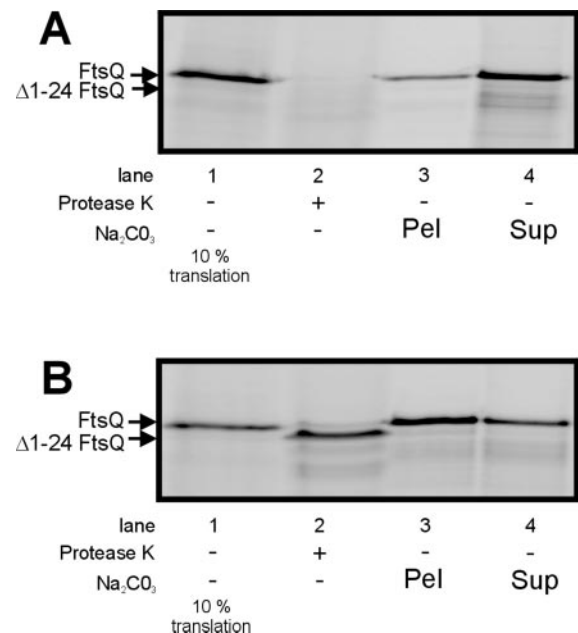


FIG. 6. The TMS of FtsQ is stably integrated into the lipid bilayer of SecYEG proteoliposomes. FtsQ insertion reactions were carried out under  $\Delta\psi$ -generating conditions in the presence of liposomes (A) or SecYEG proteoliposomes (B). A 10% translation control was removed (lane 1), residual samples were split in half, and (proteo-)liposomes were collected from the reaction mixture by centrifugation. Pellets were resuspended in either 50 mM HEPES-KOH, pH 8.0, 0.25 mg/ml proteinase K (lane 2) as translocation control or in 0.2 M  $\text{Na}_2\text{CO}_3$ , pH 11.2. After centrifugation, carbonate-extracted membrane pellets (Pel, lane 3) were resuspended in SDS sample buffer. Supernatants (Sup, lane 4) were first subjected to trichloroacetic acid precipitation.

proteins will differ in their YidC dependence and thus in the extent of their interaction with YidC. The recent observation that YidC is required for the biogenesis of  $\text{F}_1\text{F}_0$  ATPase and cytochrome *o* oxidase (30, 45) suggests that an essential function of YidC is to mediate membrane insertion and/or assembly of energy-transducing membrane protein complexes.

The reconstituted membrane protein insertion assay described here will be used to investigate the biogenesis of other Sec-dependent and -independent IMPs and the role of YidC therein.

**Acknowledgments**—We thank Corinne ten Hagen-Jongman for the construction of plasmid pBSKftsQ and Jeanine de Keyser for providing purified PrlA4 translocase.

#### REFERENCES

- Manting, E. H., and Driessen, A. J. M. (2000) *Mol. Microbiol.* **37**, 226–238
- De Gier, J. W., and Luijck, J. (2001) *Mol. Microbiol.* **40**, 314–322
- Herskovits, A. A., Bochkareva, E. S., and Bibi, E. (2000) *Mol. Microbiol.* **38**, 927–939
- Valent, Q. A., Scotti, P. A., High, S., de Gier, J. W., von Heijne, G., Lentzen, G., Wintermeyer, W., Oudega, B., and Luijck, J. (1998) *EMBO J.* **17**, 2504–2512
- Scotti, P. A., Valent, Q. A., Manting, E. H., Urbanus, M. L., Driessen, A. J. M., Oudega, B., and Luijck, J. (1999) *J. Biol. Chem.* **274**, 29883–29888
- Neumann-Haefelin, C., Schäfer, U., Müller, M., and Koch, H. G. (2000) *EMBO J.* **19**, 6419–6426
- Lee, J. I., Kuhn, A., and Dalbey, R. E. (1992) *J. Biol. Chem.* **267**, 938–943
- Andersson, H., and von Heijne, G. (1993) *EMBO J.* **12**, 683–691
- Urbanus, M. L., Scotti, P. A., Fröderberg, L., Saaf, A., de Gier, J. W., Brunner, J., Samuelson, J. C., Dalbey, R. E., Oudega, B., and Luijck, J. (2001) *EMBO Rep.* **2**, 524–529
- Urbanus, M. L., Fröderberg, L., Drew, D., Bjork, P., Scotti, P. A., de Gier, J. W., Brunner, J., Oudega, B., and Luijck, J. (2002) *J. Biol. Chem.* **277**, 12718–12723
- Scotti, P. A., Urbanus, M. L., Brunner, J., de Gier, J. W., von Heijne, G., van der Does, C., Driessen, A. J. M., Oudega, B., and Luijck, J. (2000) *EMBO J.* **19**, 542–549
- Houben, E. N., Scotti, P. A., Valent, Q. A., Brunner, J., de Gier, J. L., Oudega, B., and Luijck, J. (2000) *FEBS Lett.* **476**, 229–233
- Beck, K., Eisner, G., Trescher, D., Dalbey, R. E., Brunner, J., and Müller, M. (2001) *EMBO Rep.* **2**, 709–714
- Samuelson, J. C., Chen, M., Jiang, F., Möller, I., Wiedmann, M., Kuhn, A.,

- Phillips, G. J., and Dalbey, R. E. (2000) *Nature* **406**, 637–641
15. Brundage, L., Hendrick, J. P., Schiebel, E., Driessen, A. J. M., and Wickner, W. (1990) *Cell* **62**, 649–657
  16. van der Laan, M., Houben, E. N., Nouwen, N., Lührink, J., and Driessen, A. J. M. (2001) *EMBO Rep.* **2**, 519–523
  17. Baneyx, F., and Georgiou, G. (1990) *J. Bacteriol.* **172**, 491–494
  18. Manting, E. H., van der Does, C., Remigy, H., Engel, A., and Driessen, A. J. M. (2000) *EMBO J.* **19**, 852–861
  19. Nouwen, N., van der Laan, M., and Driessen, A. J. M. (2001) *FEBS Lett.* **508**, 103–106
  20. De Keyser, J., Van Der Does, C., Swaving, J., and Driessen, A. J. M. (2002) *FEBS Lett.* **510**, 17–21
  21. Buddelmeijer, N., Aarsman, M. E., Kolk, A. H., Vicente, M., and Nanninga, N. (1998) *J. Bacteriol.* **180**, 6107–6116
  22. Valent, Q. A., de Gier, J. W., von Heijne, G., Kendall, D. A., ten Hagen-Jongman, C. M., Oudega, B., and Lührink, J. (1997) *Mol. Microbiol.* **25**, 53–64
  23. De Vrije, T., Tommassen, J., and De Kruijff, B. (1987) *Biochim. Biophys. Acta* **900**, 63–72
  24. Cabelli, R. J., Chen, L., Tai, P. C., and Oliver, D. B. (1988) *Cell* **55**, 683–692
  25. Montoya, G., Svensson, C., Lührink, J., and Sinning, I. (1997) *Proteins* **28**, 285–288
  26. Kusters, R., Huijbregts, R., and De Kruijff, B. (1992) *FEBS Lett.* **308**, 97–100
  27. Cao, G., Kuhn, A., and Dalbey, R. E. (1995) *EMBO J.* **14**, 866–875
  28. Kiefer, D., and Kuhn, A. (1999) *EMBO J.* **18**, 6299–6306
  29. Andersson, H., and von Heijne, G. (1994) *EMBO J.* **13**, 2267–2272
  30. van der Laan, M., Urbanus, M. L., ten Hagen-Jongman, C. M., Nouwen, N., Oudega, B., Harms, N., Driessen, A. J. M., and Lührink, J. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100**, 5801–5806
  31. Valent, Q. A., Kendall, D. A., High, S., Kusters, R., Oudega, B., and Lührink, J. (1995) *EMBO J.* **14**, 5494–5505
  32. Akimaru, J., Matsuyama, S., Tokuda, H., and Mizushima, S. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 6545–6549
  33. van der Does, C., Swaving, J., van Klompenburg, W., and Driessen, A. J. M. (2000) *J. Biol. Chem.*, **275**, 2472–2478
  34. Nouwen, N., de Kruijff, B., and Tommassen, J. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 5953–5957
  35. Koch, H. G., and Müller, M. (2000) *J. Cell Biol.* **150**, 689–694
  36. Duong, F., and Wickner, W. (1999) *EMBO J.* **18**, 3263–3270
  37. Koch, H. G., Moser, M., Schimz, K. L., and Müller, M. (2002) *J. Biol. Chem.* **277**, 5715–5718
  38. Nishiyama, K., Suzuki T., and Tokuda H. (1996) *Cell* **85**, 71–81
  39. Matsumoto, G., Mori, H., and Ito K. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 13567–13572
  40. Lührink, J., ten Hagen-Jongman, C. M., van der Weijden, C. C., Oudega, B., High, S., Dobberstein, B., and Kusters, R. (1994) *EMBO J.* **13**, 2289–2296
  41. Herskovits, A. A., Seluanov, A., Rajsbaum, R., ten Hagen-Jongman, C. M., Henrichs, T., Bochkareva, E. S., Phillips, G. J., Probst, F. J., Nakae, T., Ehrmann, M., Lührink, J., and Bibi, E. (2001) *EMBO Rep.* **2**, 1040–1046
  42. Samuelson, J. C., Jiang, F., Yi, L., Chen, M., de Gier, J. W., Kuhn, A., and Dalbey, R. E. (2001) *J. Biol. Chem.* **276**, 34847–34852
  43. Chen, M., Xie, K., Nouwen, N., Driessen, A. J. M., and Dalbey, R. E. (2003) *J. Biol. Chem.* **278**, 23295–23300
  44. Kuhn, A., Stuart, R., Henry, R., and Dalbey, R. E. (2003) *Trends Cell Biol.* **13**, 510–516
  45. Yi, L., Jiang, F., Chen, M., Cain, B., Bolhuis, A., and Dalbey, R. E. (2003) *Biochemistry* **42**, 10537–10544